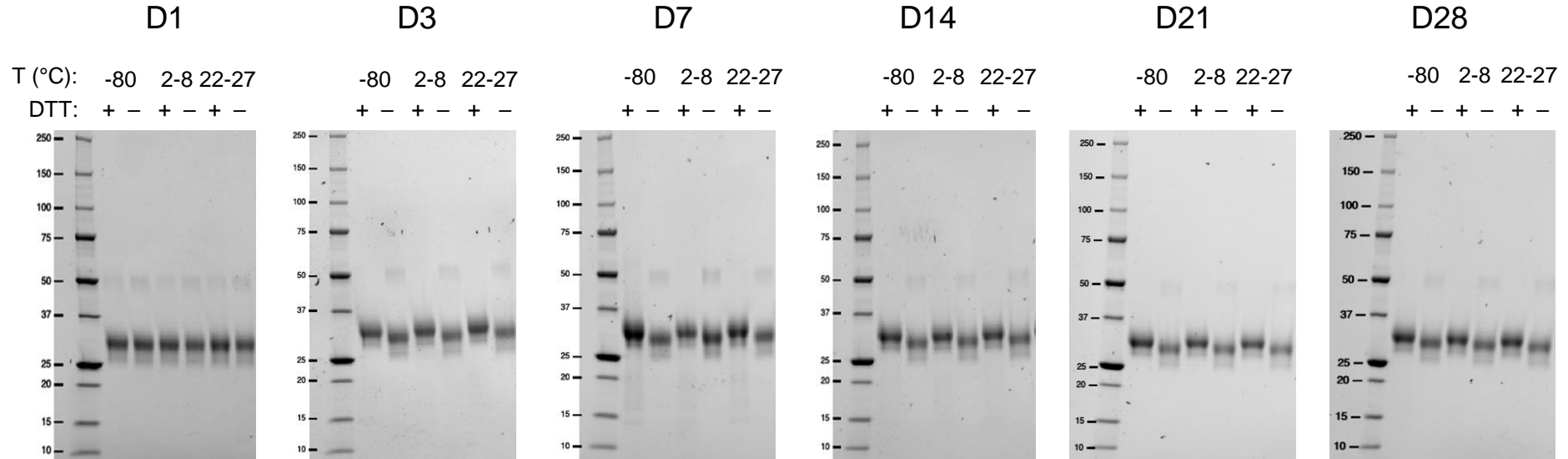


Elicitation of potent neutralizing antibody responses by designed protein nanoparticle vaccines for SARS-CoV-2

Walls AC & Fiala B, et al.

Supplemental Stability Data

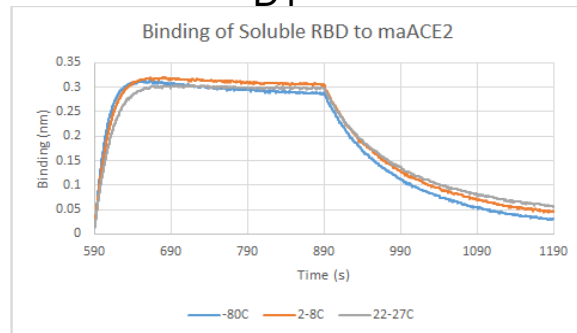
SDS-PAGE for Monomeric RBD



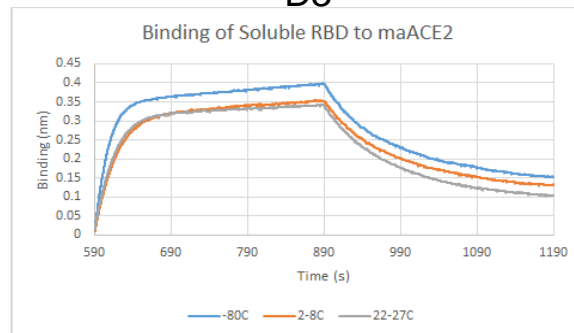
The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT.

mACE2-Fc Binding for Monomeric RBD

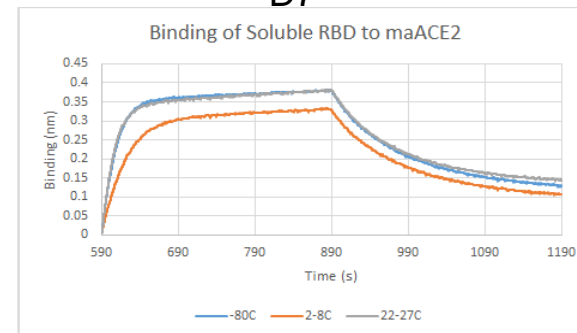
D1



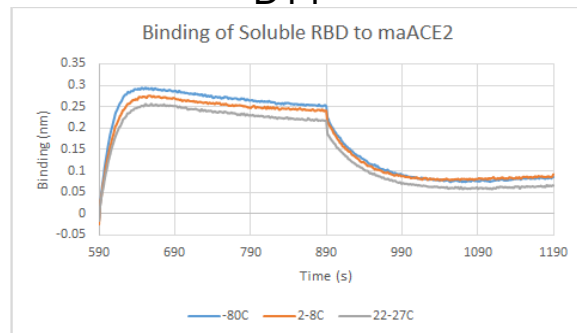
D3



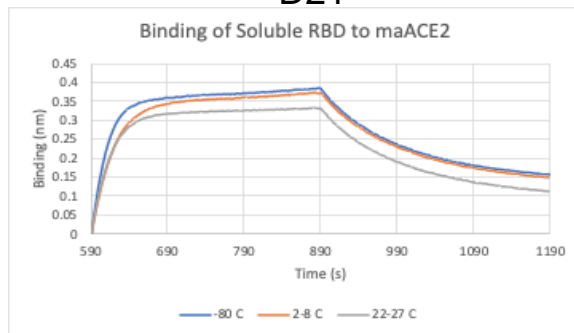
D7



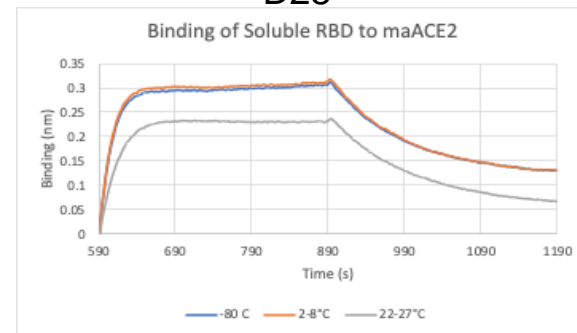
D14



D21



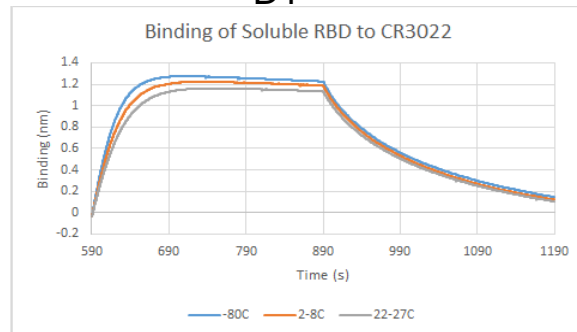
D28



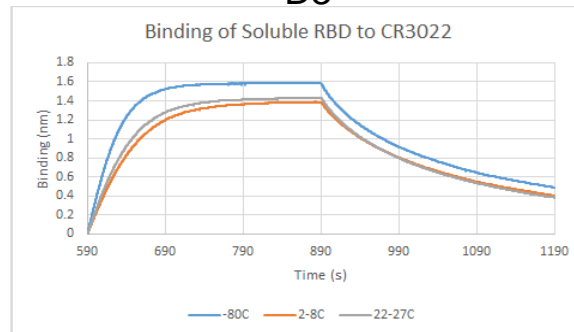
Binding of antigen incubated at three different temperatures for various numbers of days (D) to mACE2-Fc was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with mACE2-Fc were incubated with antigen (association, x = 590–889 s) and then buffer (dissociation, x = 890–1190 s).

CR3022 IgG Binding for Monomeric RBD

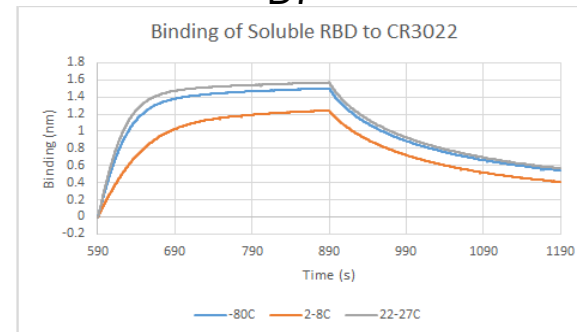
D1



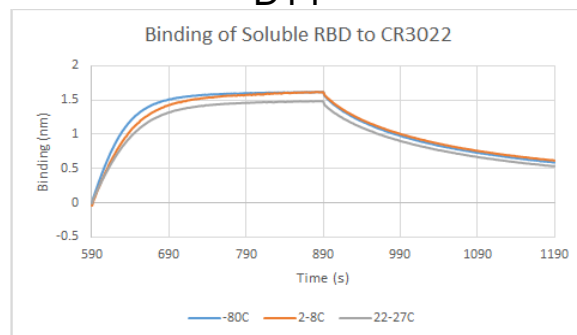
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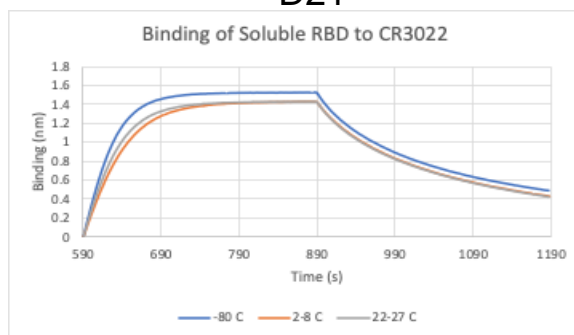
D7



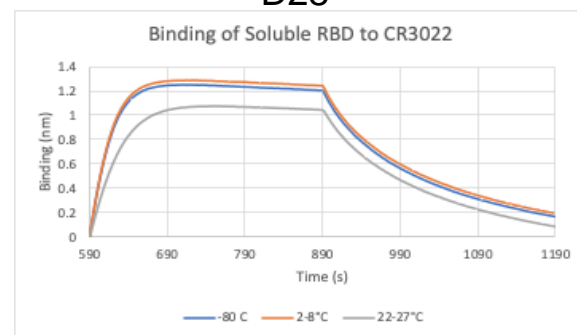
D14



D21

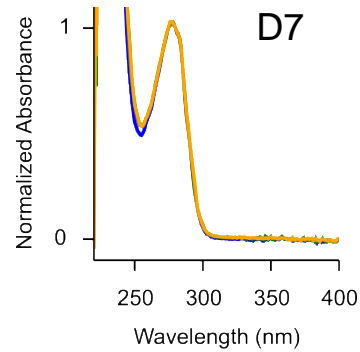
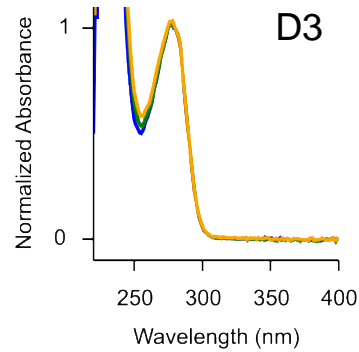
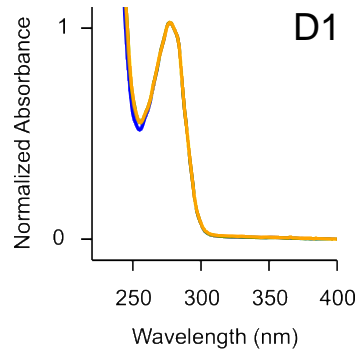


D28

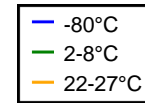
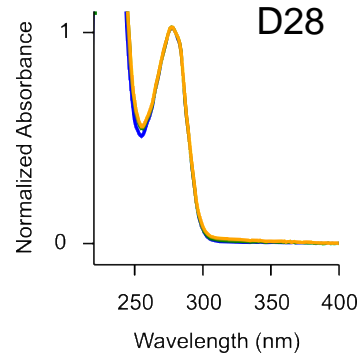
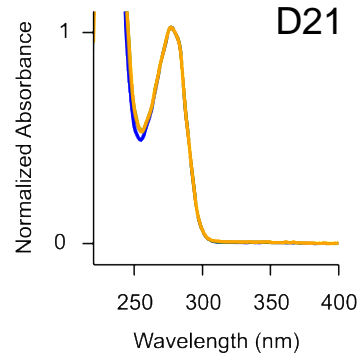
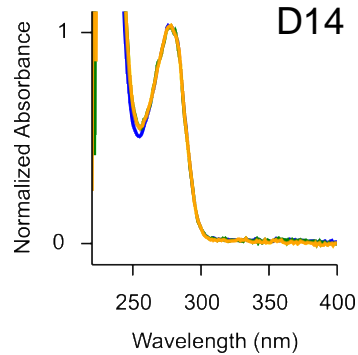


Binding of antigen incubated at three different temperatures for various numbers of days (D) to CR3022 was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with CR3022 were incubated with antigen (association, x = 590–889 s) and then buffer (dissociation, x = 890–1190 s).

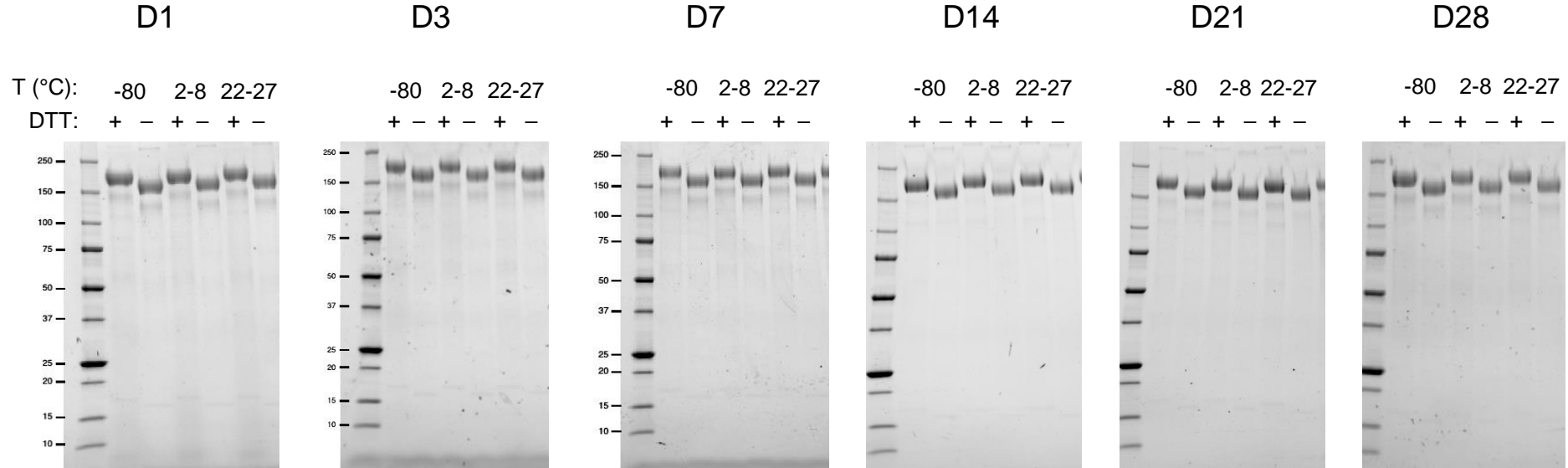
Absorbance at 320/280 nm for Monomeric RBD



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.

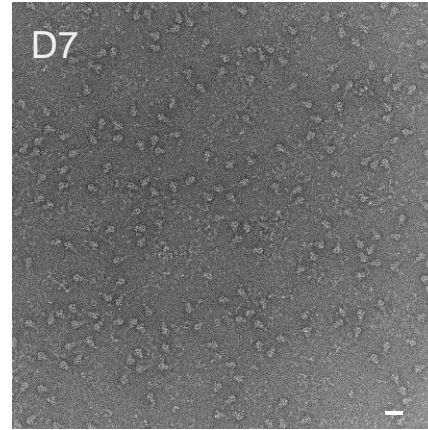
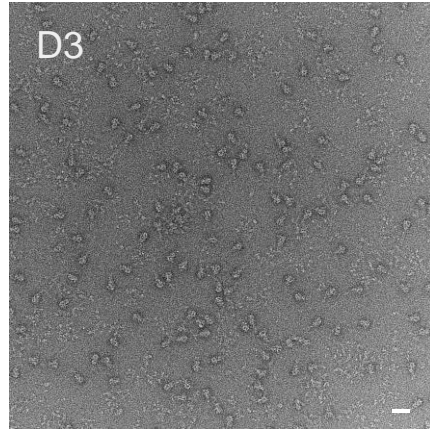
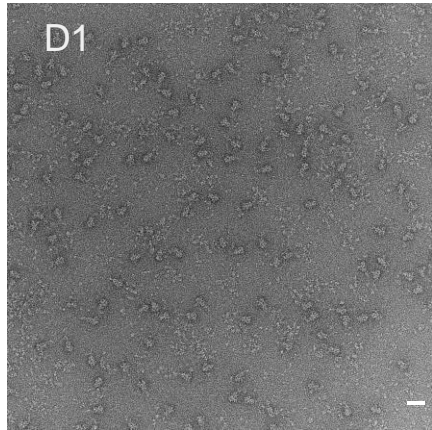


SDS-PAGE for S-2P trimer

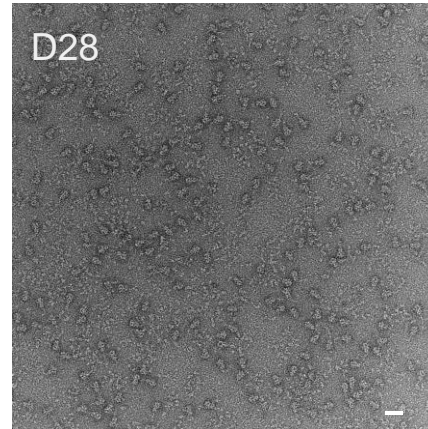
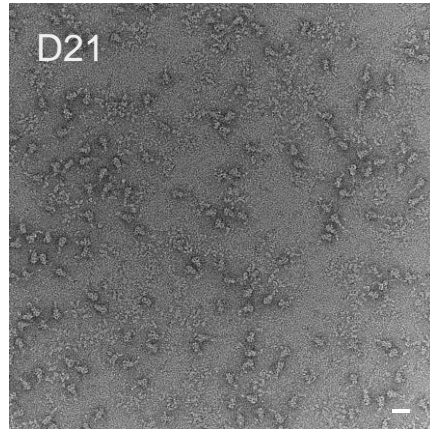
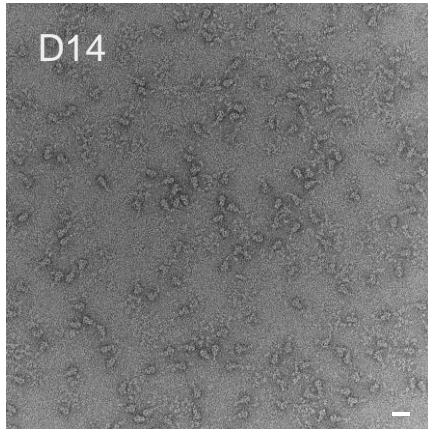


The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT.

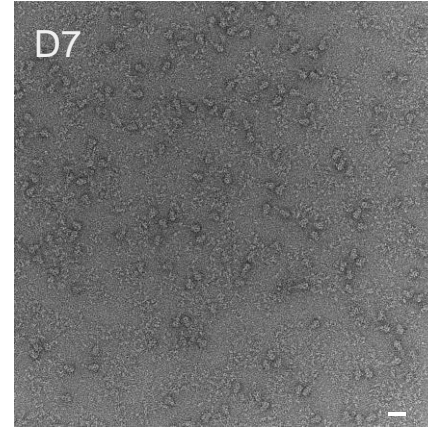
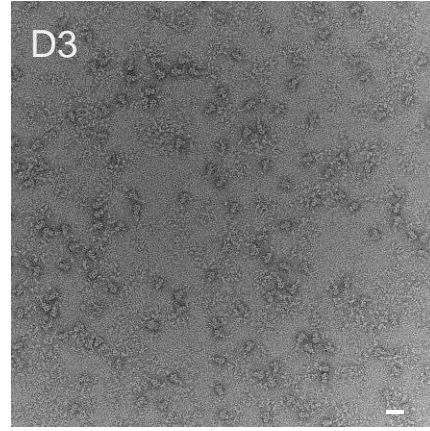
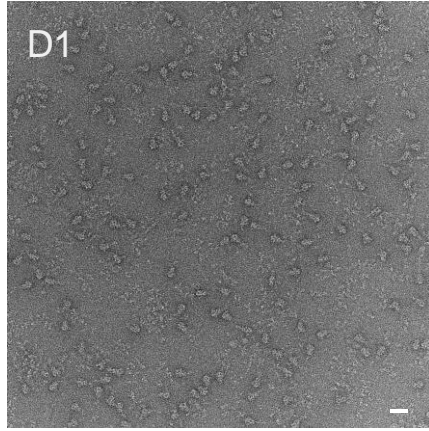
nsEM for S-2P Trimer, -80°C



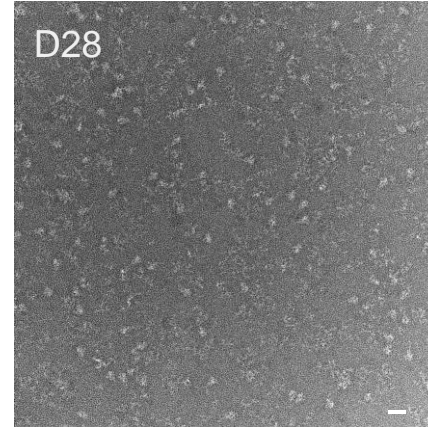
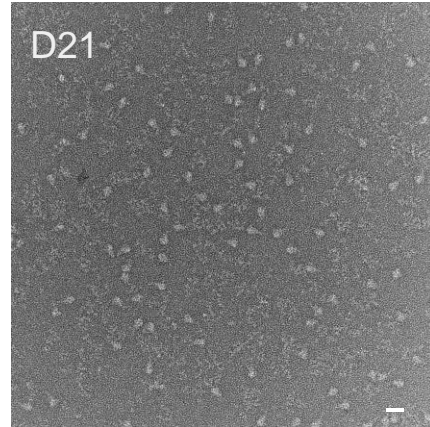
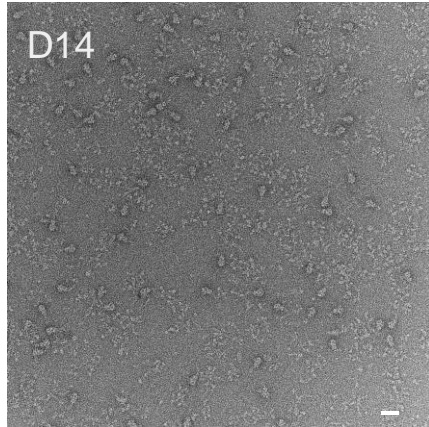
Representative negative stain electron micrographs for each sample at each time point (D, day). Scale bar, 25 nm.



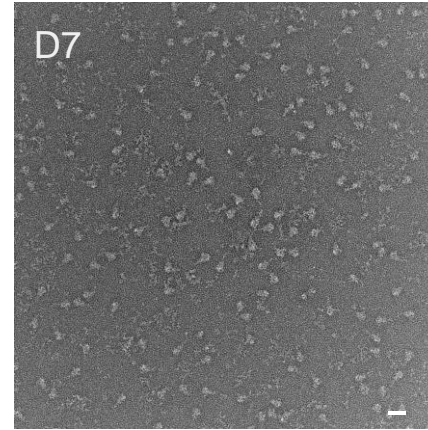
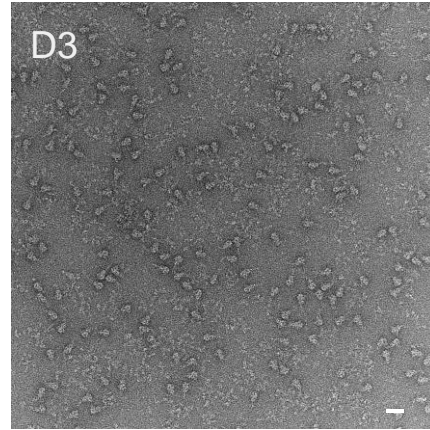
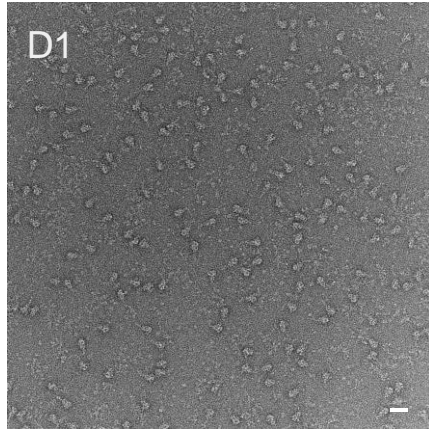
nsEM for S-2P Trimer, 2-8°C



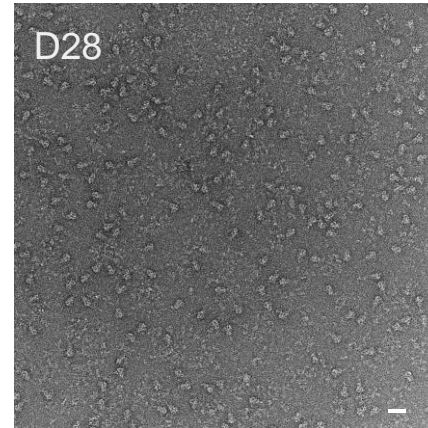
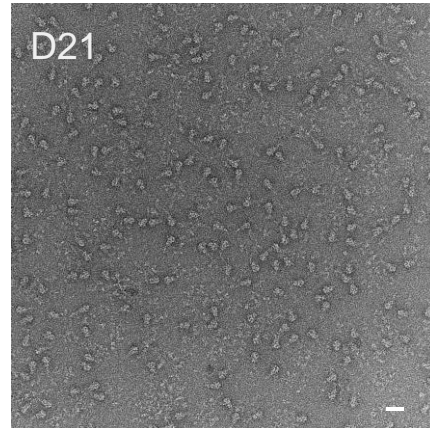
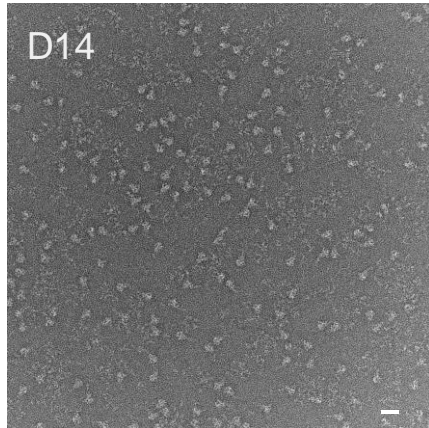
Representative negative stain electron micrographs for each sample at each time point (D, day). Scale bar, 25 nm.



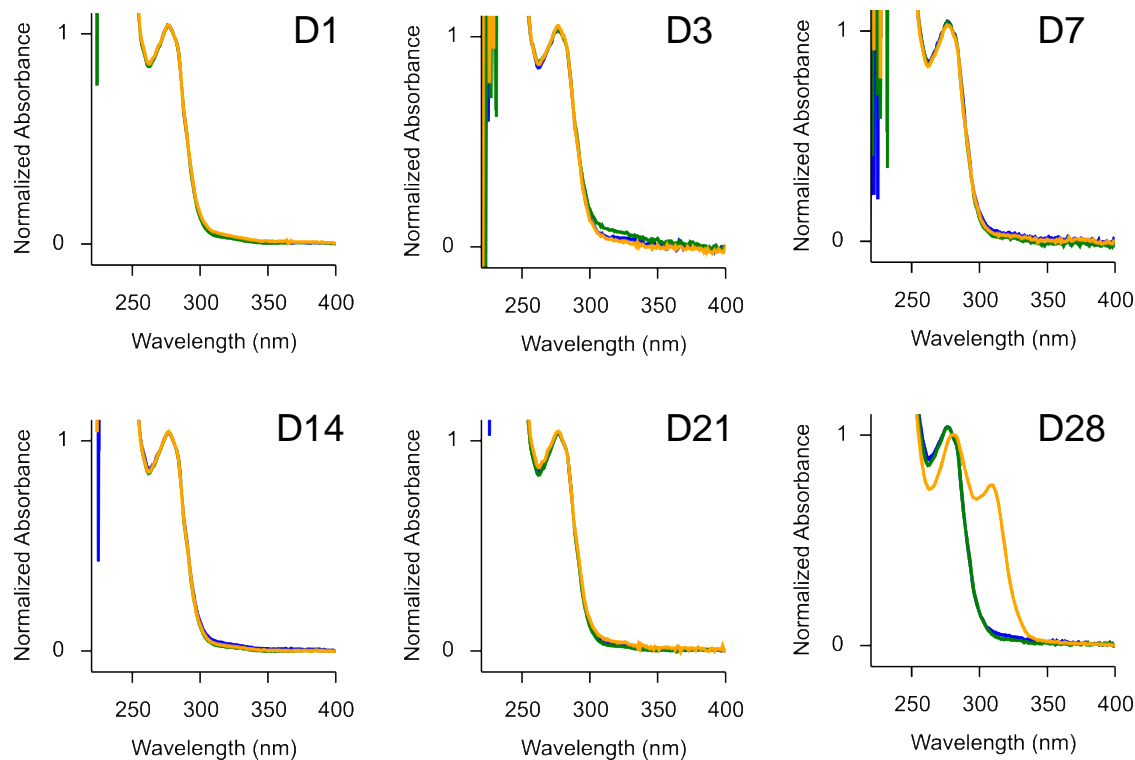
nsEM for S-2P Trimer, 22-27°C



Representative negative stain electron micrographs for each sample at each time point (D, day). Scale bar, 25 nm.

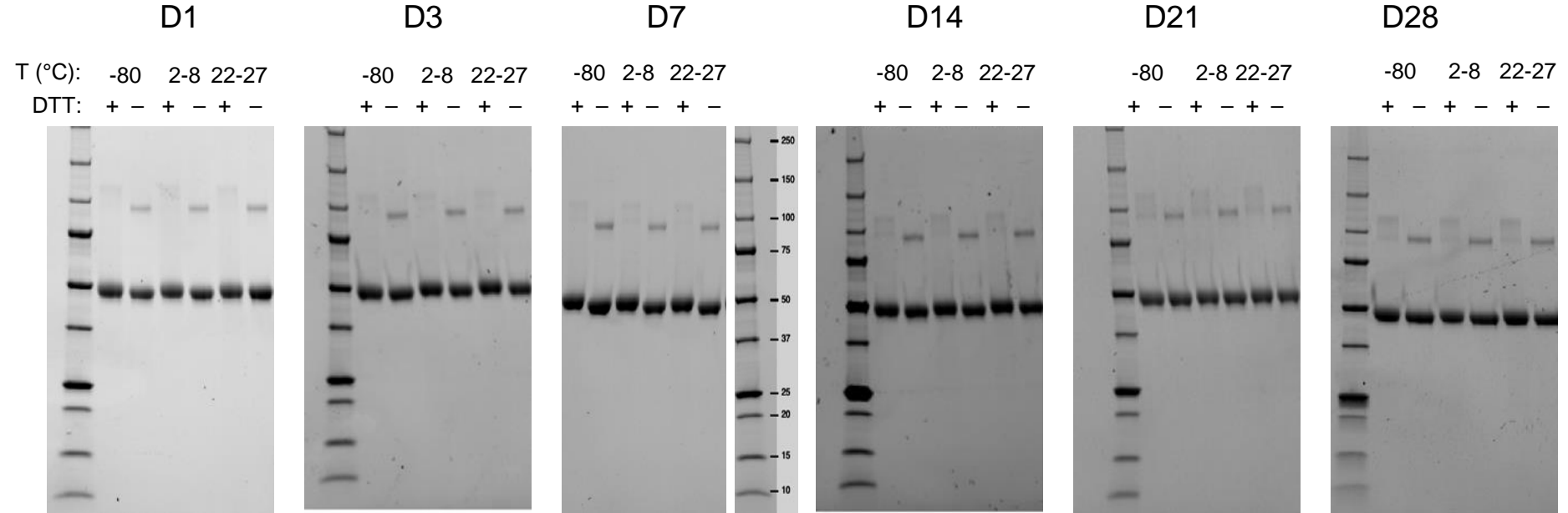


Absorbance at 320/280 for S-2P Trimer



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.

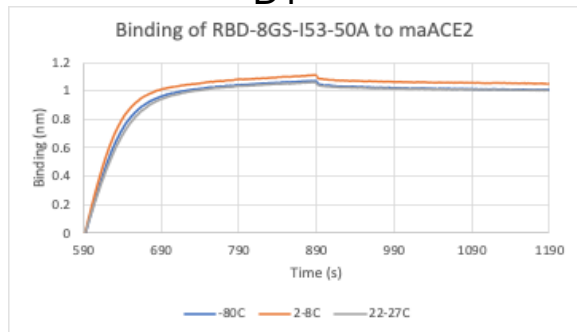
SDS-PAGE for RBD-8GS-I53-50A component



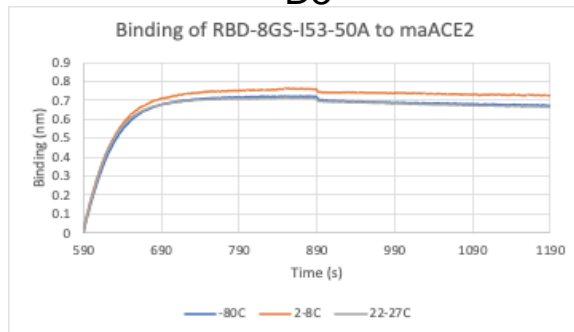
The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT.

mACE2-Fc Binding for RBD-8GS-I53-50A component

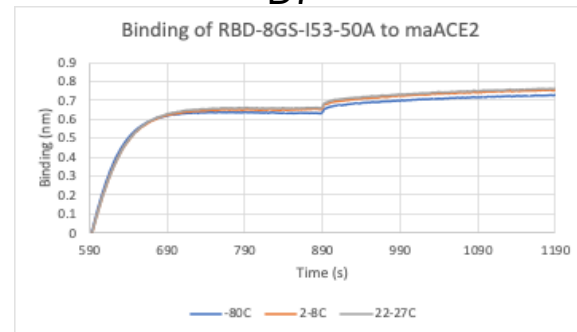
D1



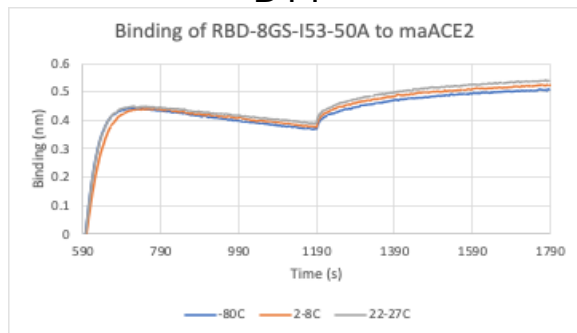
D3



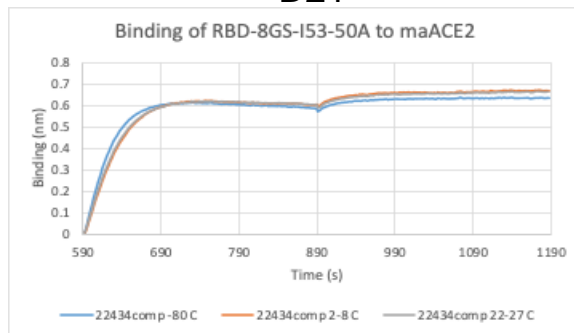
D7



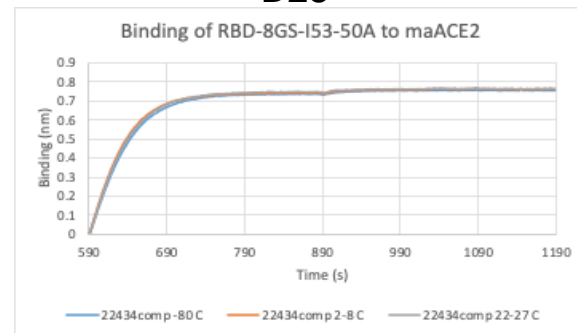
D14



D21



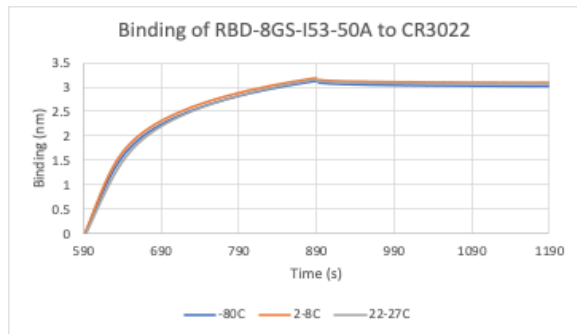
D28



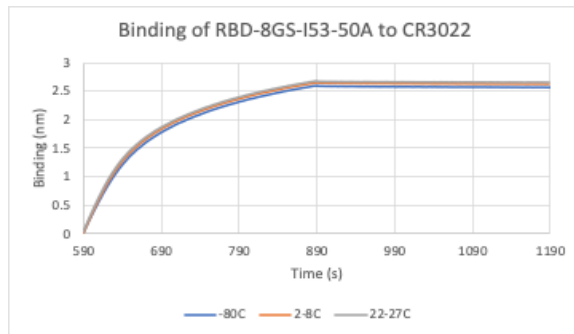
Binding of antigen incubated at three different temperatures for various numbers of days (D) to mACE2-Fc was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with mACE2-Fc were incubated with antigen (association, $x = 590-889$ s) and then buffer (dissociation, $x = 890-1190$ s).

CR3022 IgG for RBD-8GS-I53-50A component

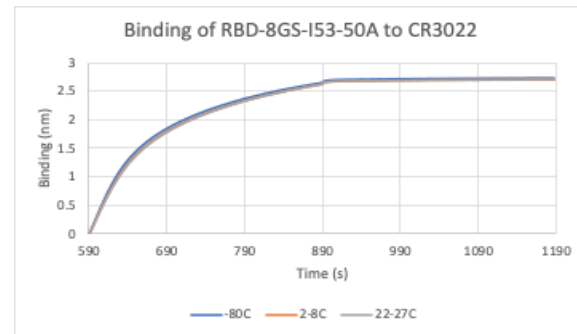
D1



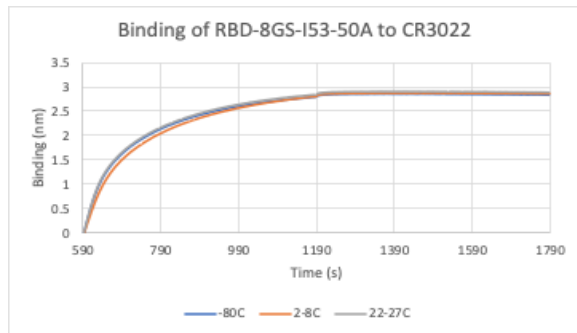
D3



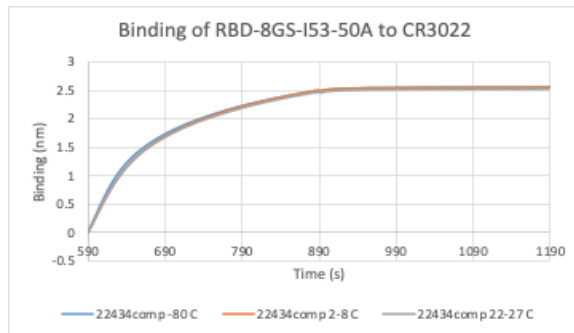
D7



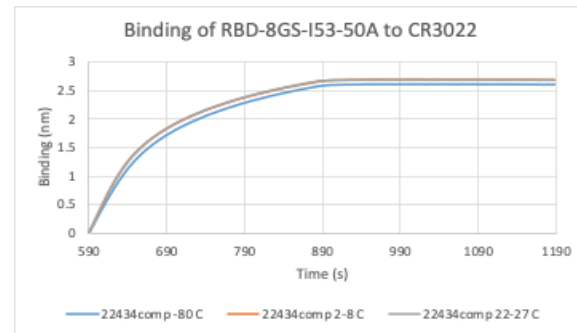
D14



D21

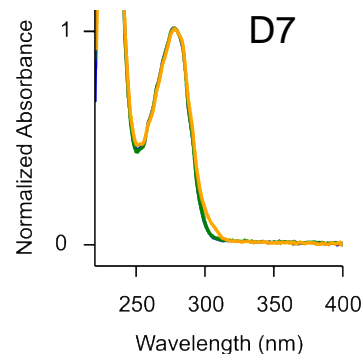
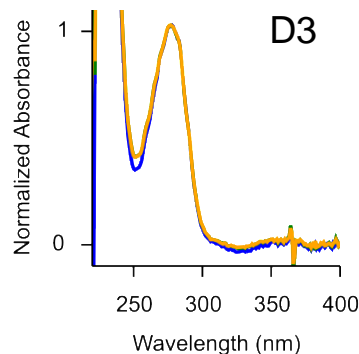
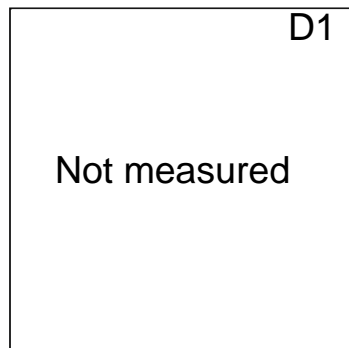


D28

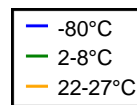
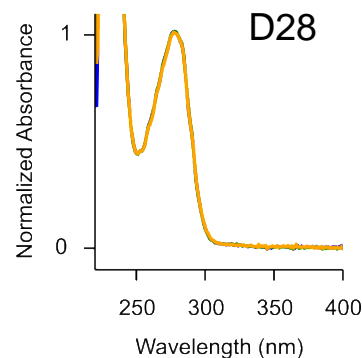
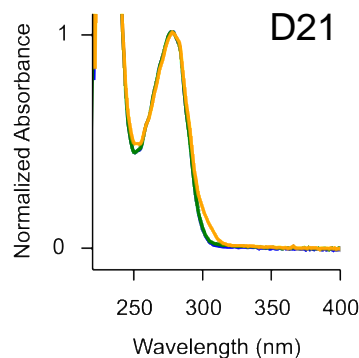
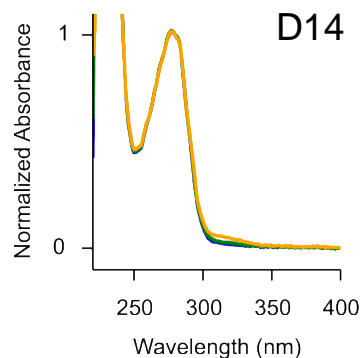


Binding of antigen incubated at three different temperatures for various numbers of days (D) to CR3022 was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with CR3022 were incubated with antigen (association, x = 590–889 s) and then buffer (dissociation, x = 890–1190 s).

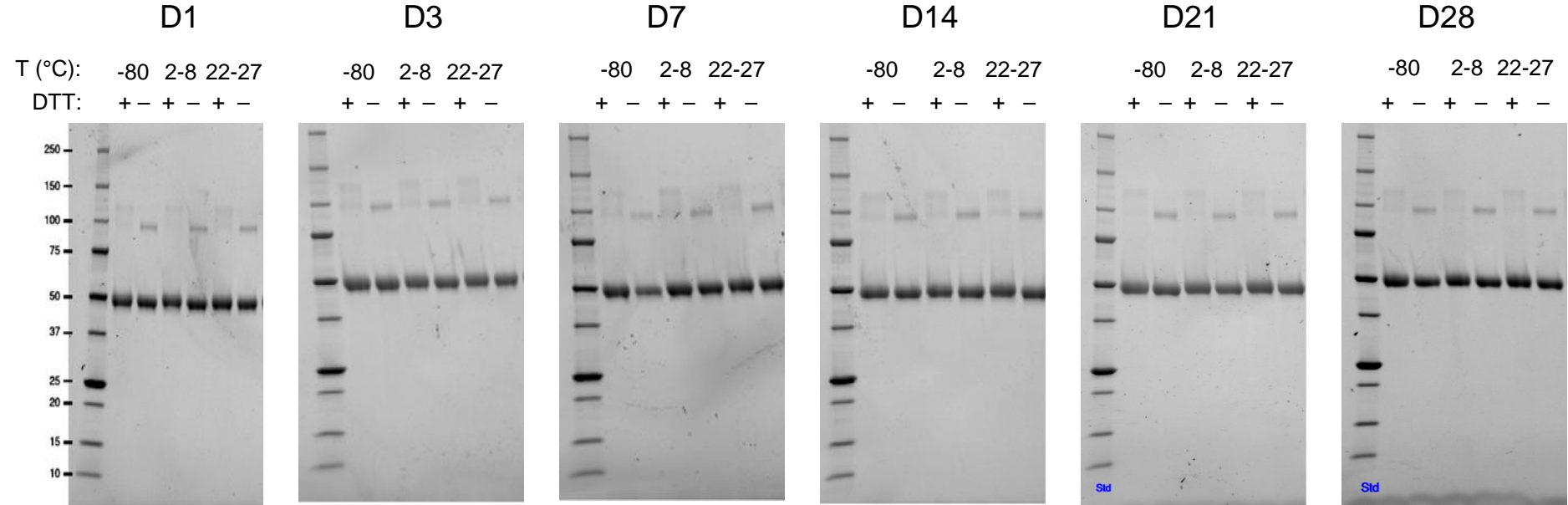
Absorbance at 320/280 for RBD-8GS-I53-50A component



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.



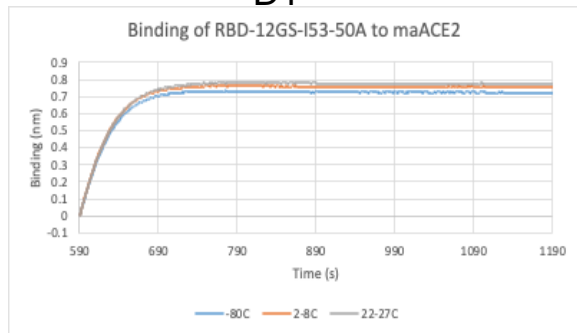
SDS-PAGE for RBD-12GS-I53-50A component



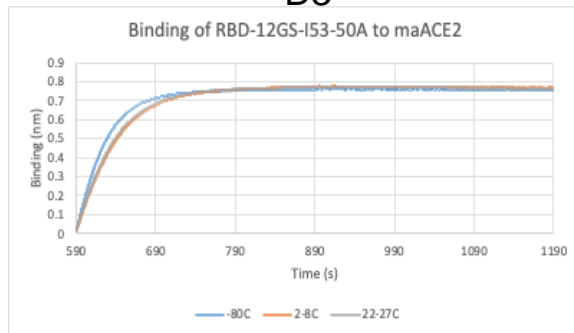
The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT.

mACE2-Fc Binding for RBD-12GS-I53-50A component

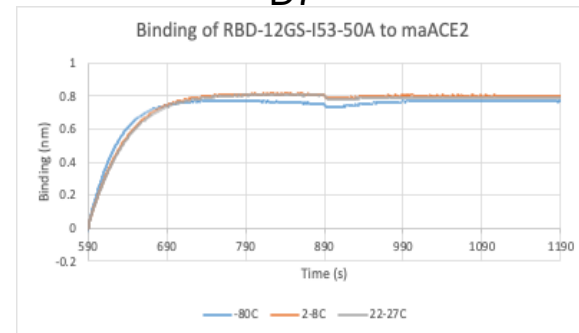
D1



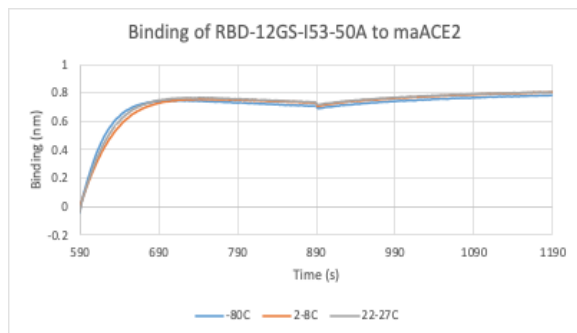
D3



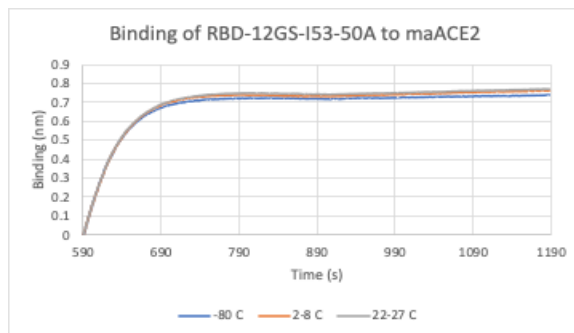
D7



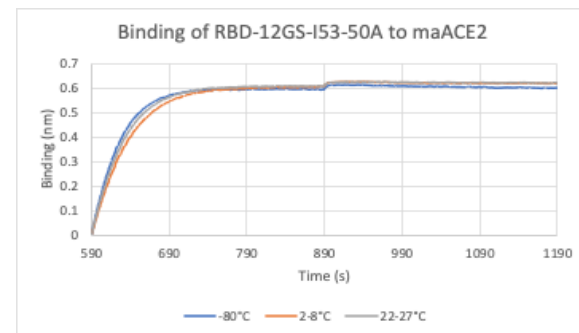
D14



D21



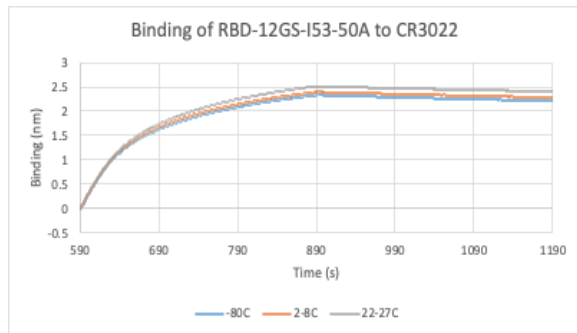
D28



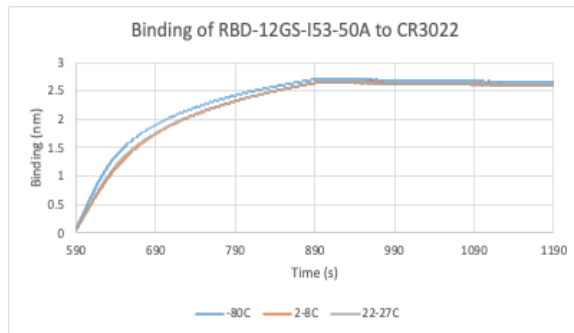
Binding of antigen incubated at three different temperatures for various numbers of days (D) to mACE2-Fc was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with mACE2-Fc were incubated with antigen (association, $x = 590-889$ s) and then buffer (dissociation, $x = 890-1190$ s).

CR3022 IgG Binding for RBD-12GS-I53-50A component

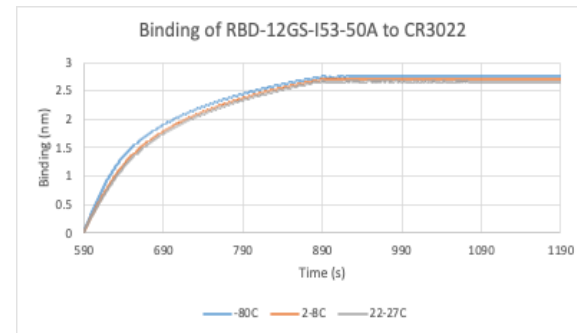
D1



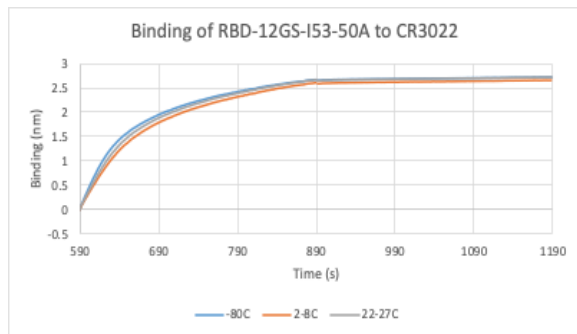
D3



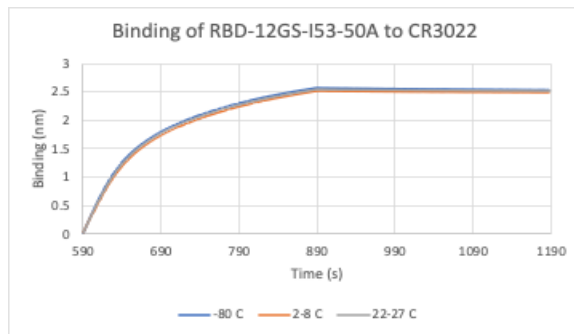
D7



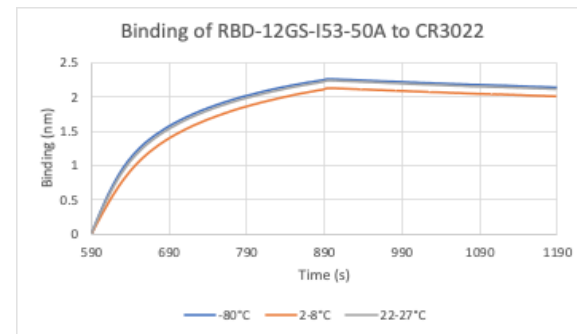
D14



D21

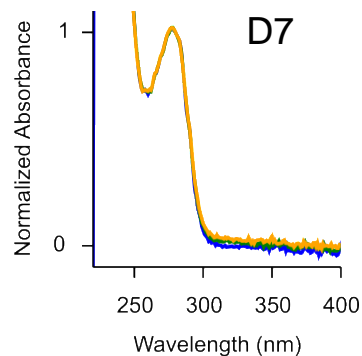
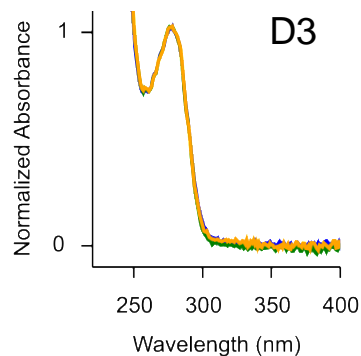
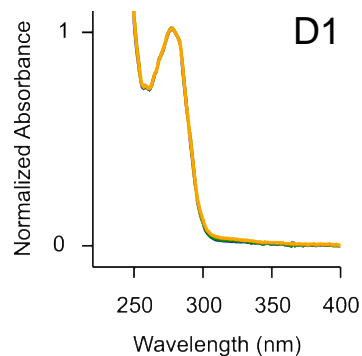


D28

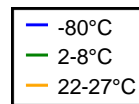
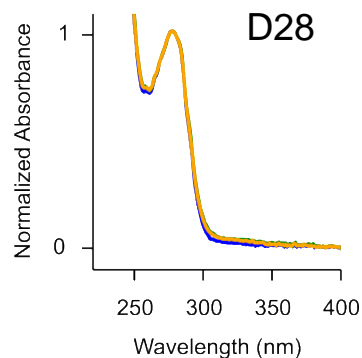
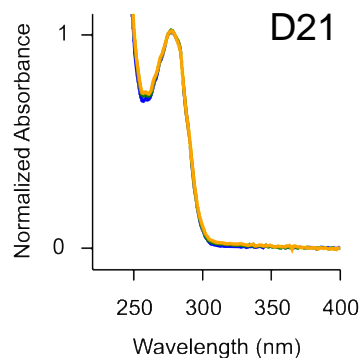
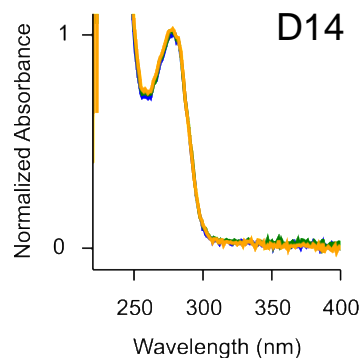


Binding of antigen incubated at three different temperatures for various numbers of days (D) to CR3022 was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with CR3022 were incubated with antigen (association, x = 590–889 s) and then buffer (dissociation, x = 890–1190 s).

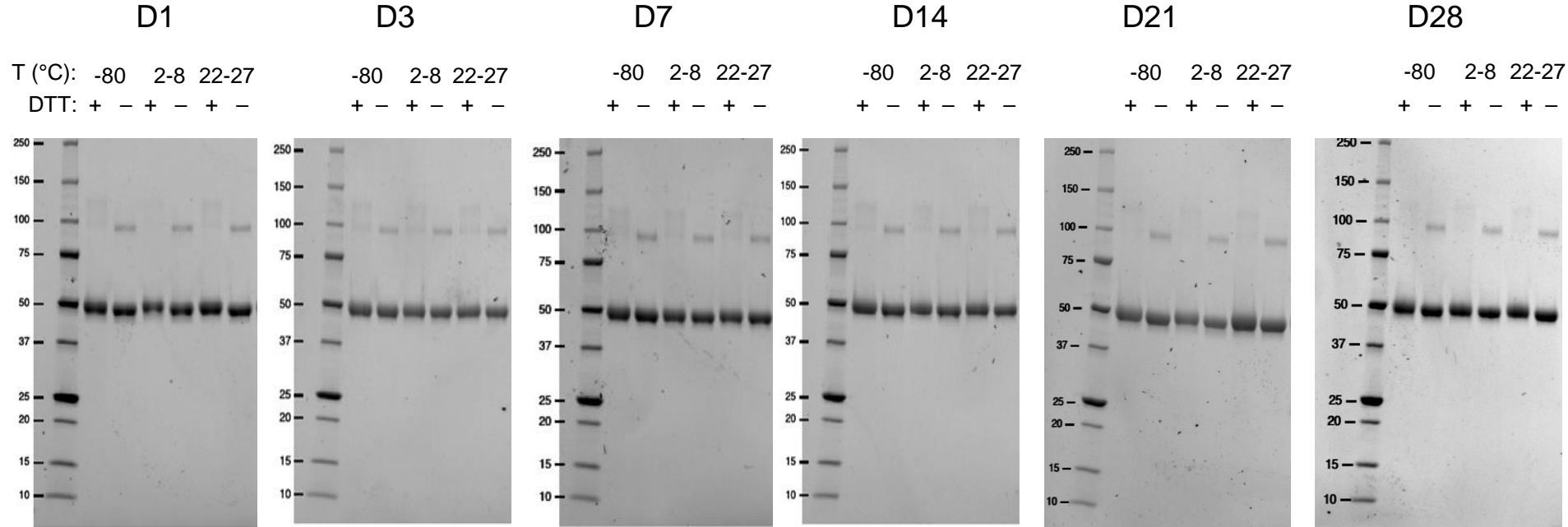
Absorbance at 320/280 nm for RBD-12GS-I53-50A component



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.



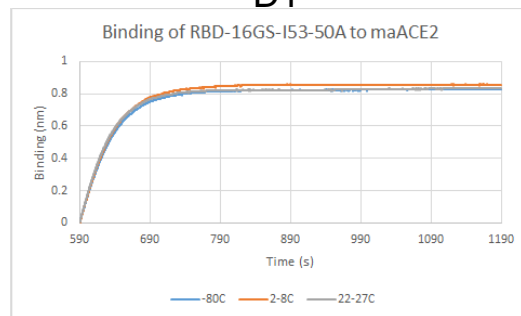
SDS-PAGE for RBD-16GS-I53-50A component



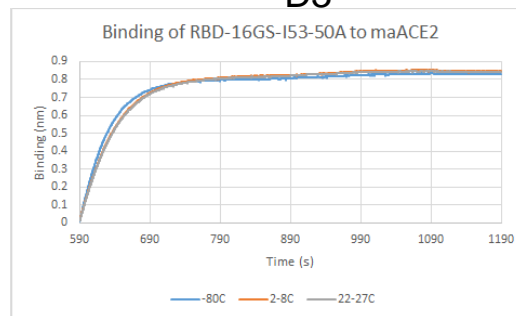
The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT.

mACE2-Fc Binding for RBD-16GS-I53-50A component

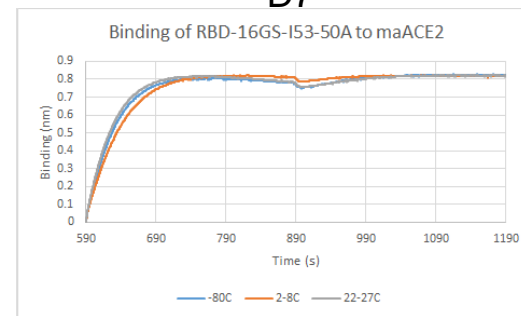
D1



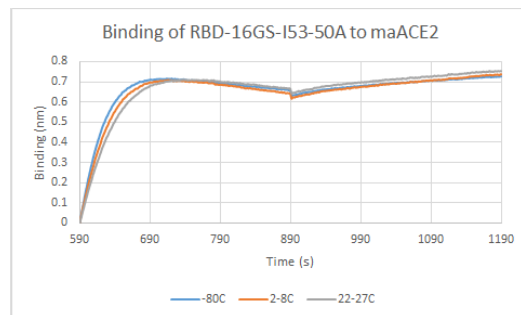
D3



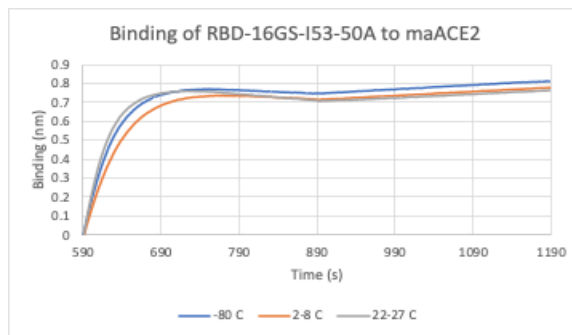
D7



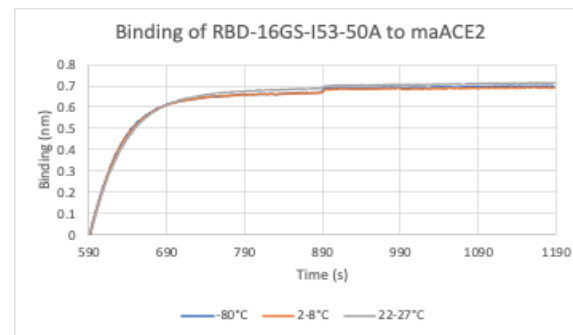
D14



D21



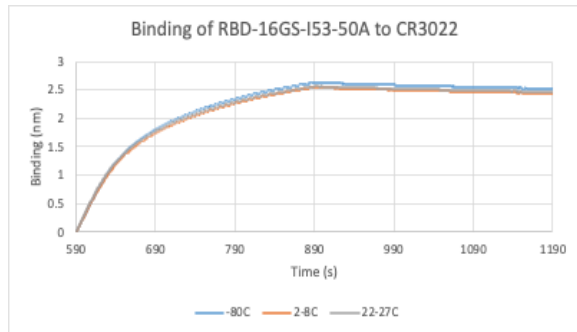
D28



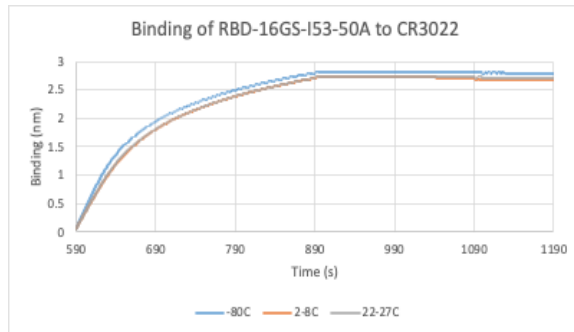
Binding of antigen incubated at three different temperatures for various numbers of days (D) to mACE2-Fc was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with mACE2-Fc were incubated with antigen (association, $x = 590-889$ s) and then buffer (dissociation, $x = 890-1190$ s).

CR3022 IgG Binding for RBD-16GS-I53-50A component

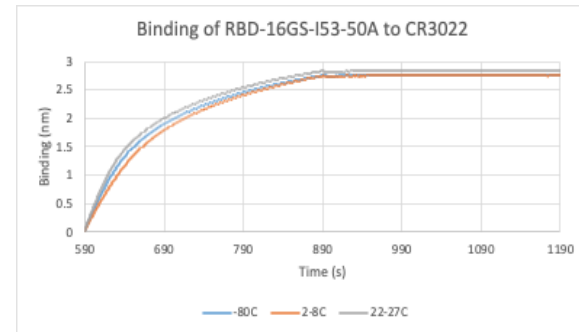
D1



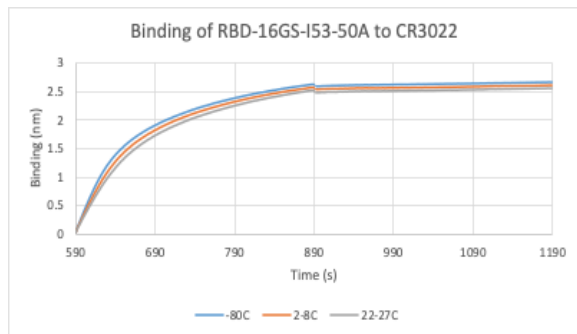
D3



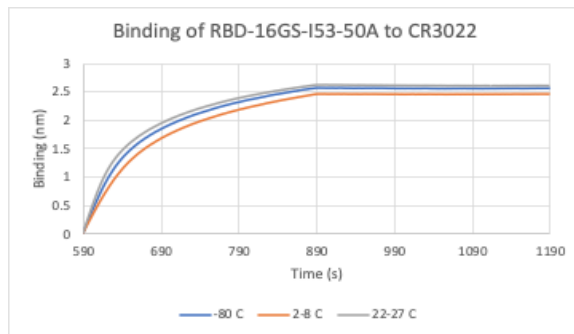
D7



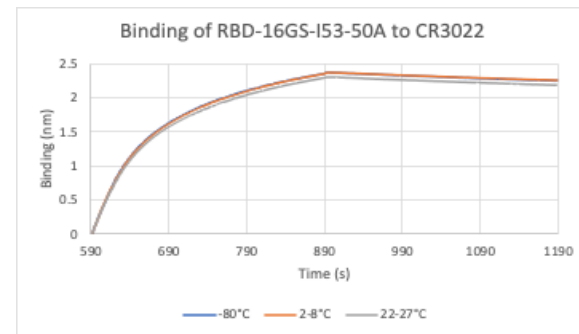
D14



D21

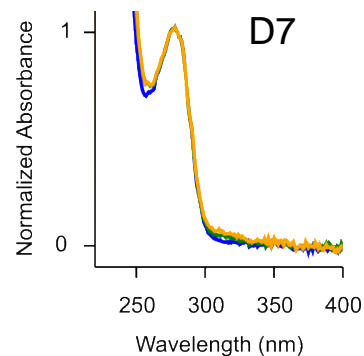
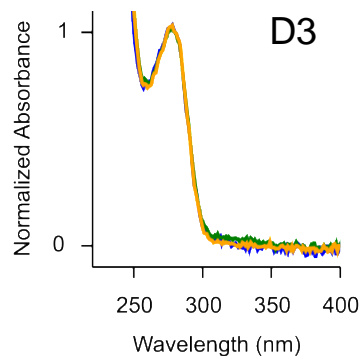
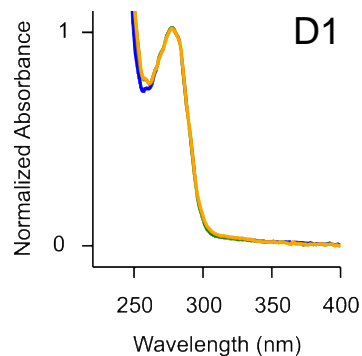


D28

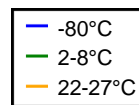
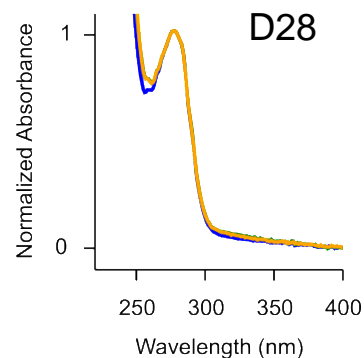
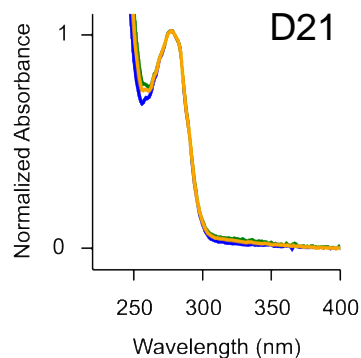
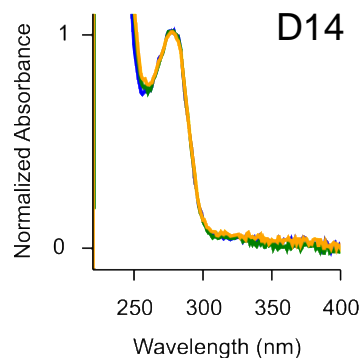


Binding of antigen incubated at three different temperatures for various numbers of days (D) to CR3022 was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with CR3022 were incubated with antigen (association, $x = 590-889$ s) and then buffer (dissociation, $x = 890-1190$ s).

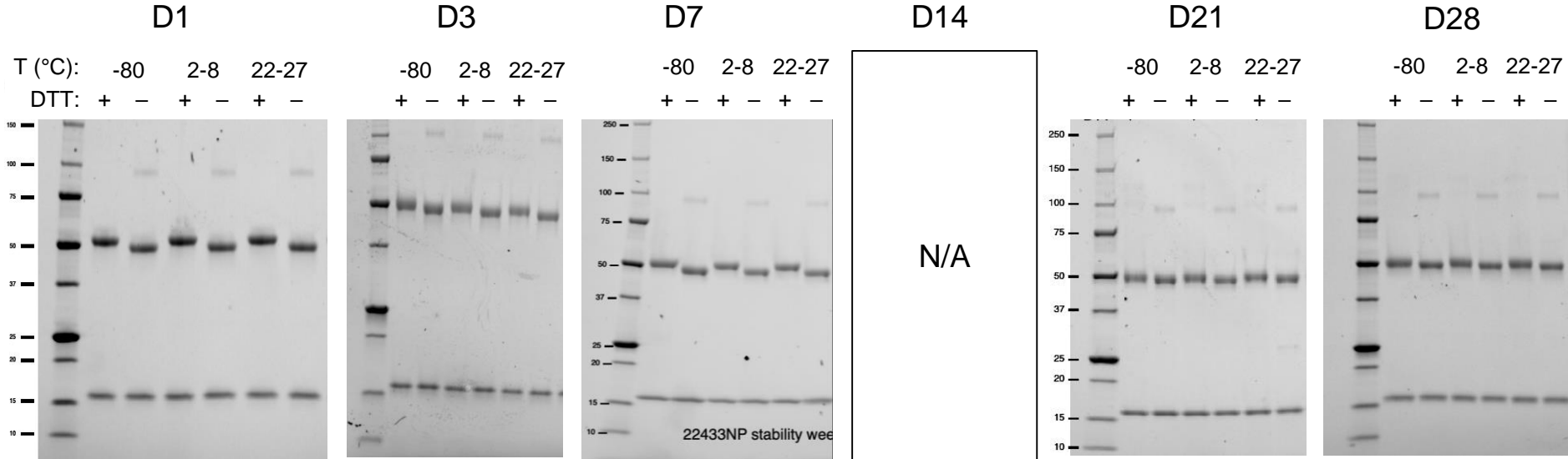
Absorbance at 320/280 for RBD-16GS-I53-50A component



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.



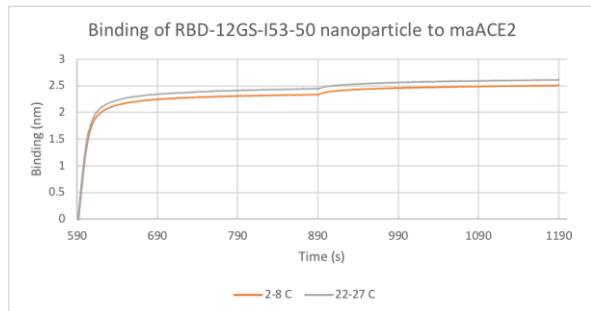
SDS-PAGE for RBD-12GS-I53-50 nanoparticle



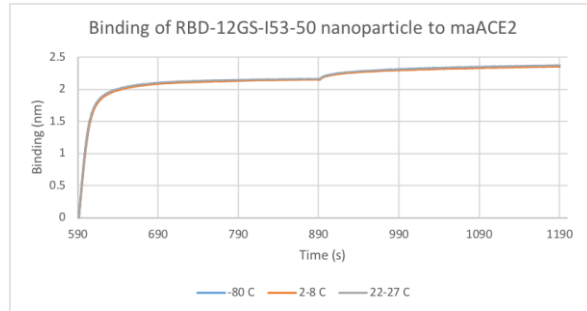
The integrity of samples incubated at three different temperatures for various numbers of days (D) was analyzed by SDS-PAGE. Molecular weights of the standard are noted in kDa. Each sample was analyzed +/- DTT. N/A: Not assessed.

mACE2-Fc Binding for RBD-12GS-I53-50 nanoparticle

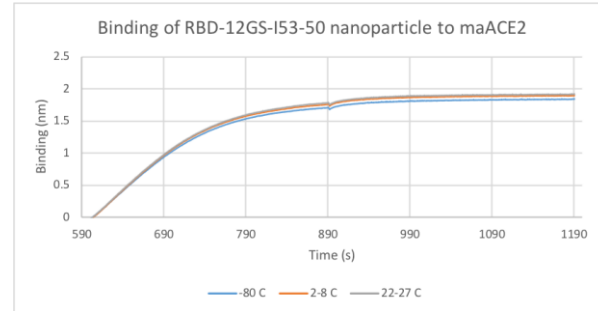
D1



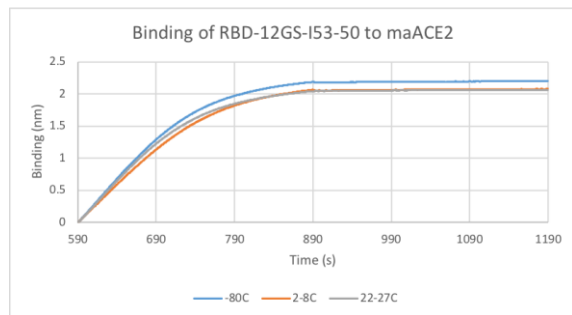
D3



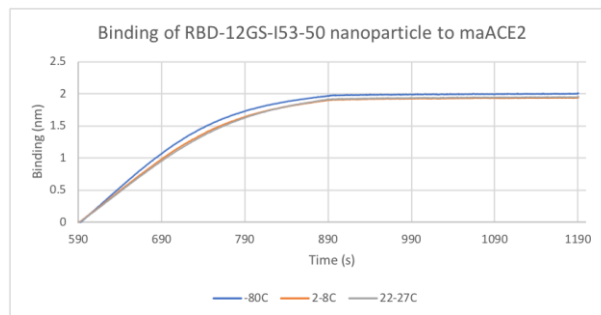
D7



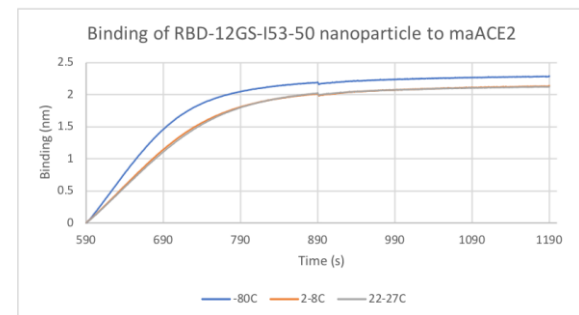
D14



D21



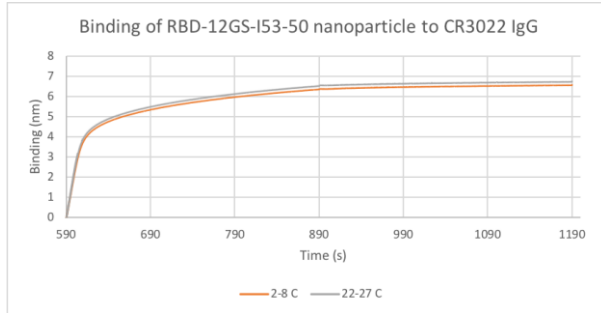
D28



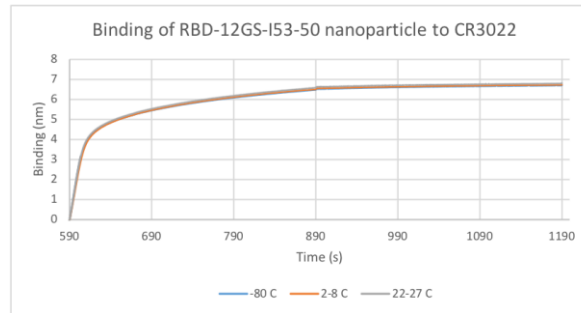
Binding of antigen incubated at three different temperatures for various numbers of days (D) to mACE2-Fc was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with mACE2-Fc were incubated with antigen (association, $x = 590-889$ s) and then buffer (dissociation, $x = 890-1190$ s).

CR3022 IgG Binding for RBD-12GS-I53-50 nanoparticle

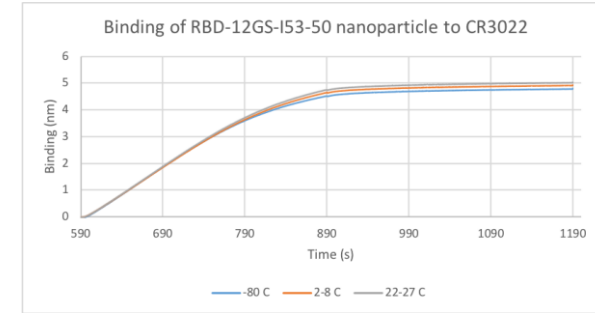
D1



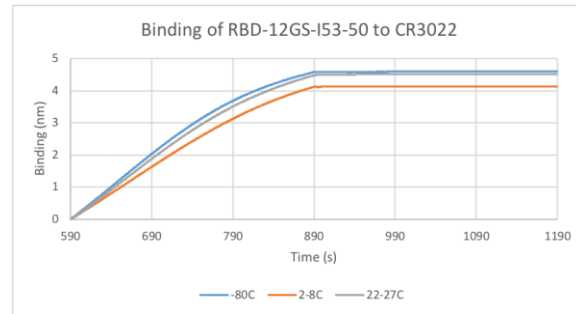
D3



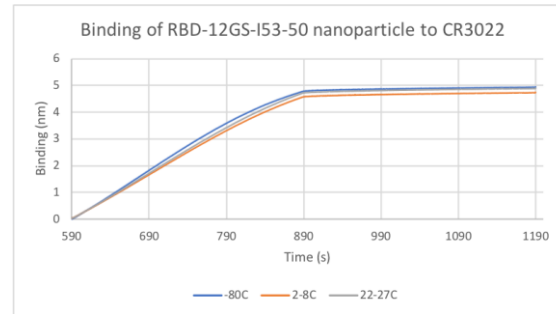
D7



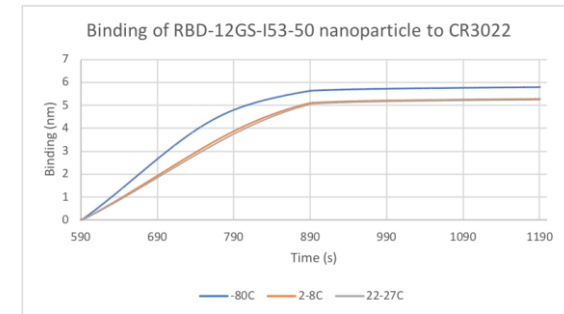
D14



D21

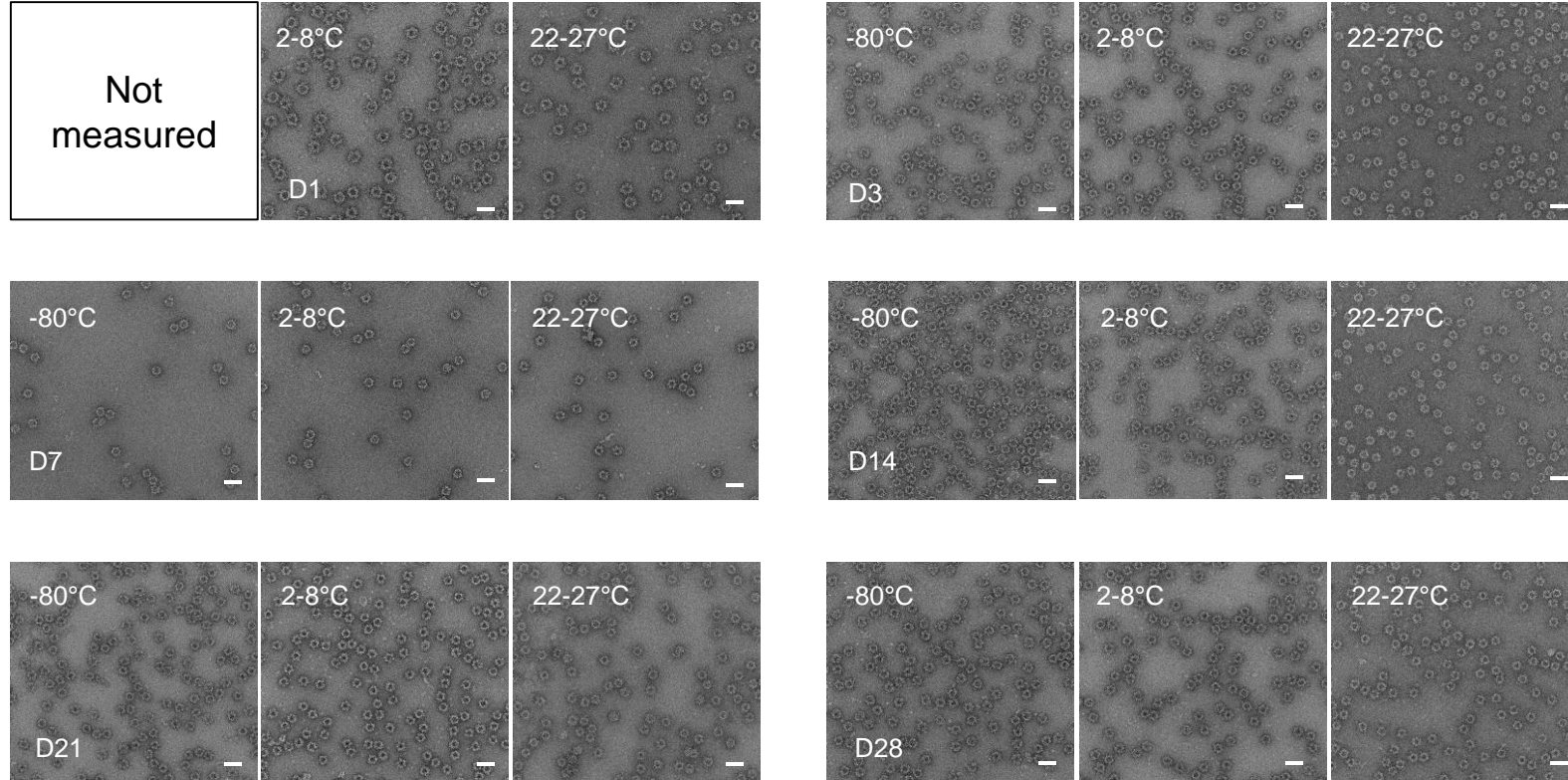


D28



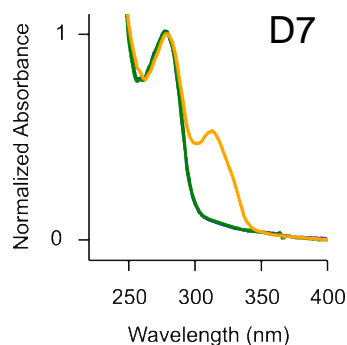
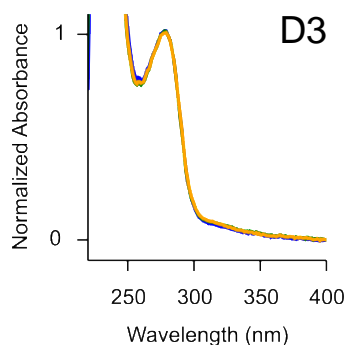
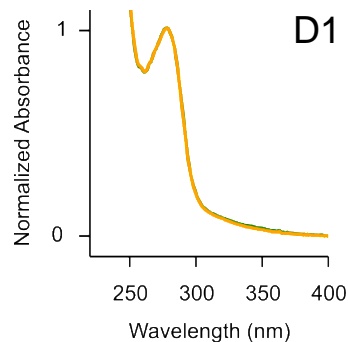
Binding of antigen incubated at three different temperatures for various numbers of days (D) to CR3022 was analyzed by Biolayer Interferometry (BLI). Protein A biosensors loaded with CR3022 were incubated with antigen (association, x = 590–889 s) and then buffer (dissociation, x = 890–1190 s).

nsEM for RBD-12GS-I53-50 nanoparticle



Representative negative stain electron micrographs for each sample at each time point (D, day). Scale bar, 50 nm.

Absorbance at 320/280 nm for RBD-12GS-I53-50 nanoparticle



UV/vis absorbance spectra for each sample at each time point were obtained on an Agilent Cary 8454 and normalized such that $A_{280} = 1$ and $A_{400} = 0$.

